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ADULT AND LARVAL STAGES OF *PARAUSTROSTRONGYLUS RATTI* (NEMATODA: TRICHOSTRONGYLOIDEA) FROM *RATTUS FUSCIPES*

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Summary

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The morphology of the adult and the third and fourth larval stages of *Paraustrostrongylus ratti* from the native rodent *Rattus fuscipes* is described, with particular reference to the synlophe, as well as the mechanism of attachment of the nematode to intestinal villi. The synlophe of the fourth larval stage has an oblique axis of orientation, in contrast to the frontal orientation in the adult stage, and most closely resembles the synlophe of species of *Dessetostrongylus* parasitic in dasyurid marsupials. Ontogenetic data therefore suggest that *Paraustrongylus* evolved from an ancestor resembling *Dessetostrongylus*.

KEY WORDS: Nematodes, Trichostrongyloidea, *Paraustrostrongylus*, ontogenesis, larvae, morphology, rodents.

Introduction

The trichostrongyloid nematode subfamily Herpetostrongylinae occurs in the small intestines of Australian marsupials and is one of the few trichostrongyloid groups in which there is apparently a close evolutionary parallel between hosts and parasites (Durette-Desset 1982, 1985; Humphery-Smith 1983; Beveridge 1986). Three distinct lineages have been recognised among the eight component genera in marsupials, each sharing a probable common ancestry with *Woolleya*, a contemporary genus which occurs in dasyurid marsupials. *Woolleya* shares features in common with the genus *Viannaia*, belonging to the family Viannaiidae, which is present in South American marsupials and rodents (Humphery-Smith 1983).

Two of the herpetostrongyline genera, *Austrostrongylus* and *Paraustrostrongylus*, occurring in diprotodont marsupials, with one species in the marsupial mole, *Notoryctes typhlops*, and one in a rodent, *Rattus fuscipes*, are of particular morphological interest due to the development of paired lateral cuticular inflations, unique within the Trichostrongyloidea, termed "floats" by Durette-Desset (1979). The evolutionary development of these floats was investigated in *Austrostrongylus* and in a related genus, *Sutarostrongylus*, (Beveridge & Durette-Desset 1986) and species with intermediate or primitive morphological features, that is either with a single float or without floats, were identified. This study suggested that *Sutarostrongylus*, parasitic in *Thylogale* spp., exhibited a number of primitive characters, ancestral to those seen in *Austrostrongylus*, but provided no additional insights into the possible relationships between a postulated *Woolleya*-like ancestor and *Sutarostrongylus*. *Dessetostrongylus* is one possible intermediary between *Woolleya* and *Sutarostrongylus* (see Beveridge & Durette-Desset 1986) as it has a synlophe, or complement of body ridges, identical with that of *Sutarostrongylus* except for the fact that the axis of orientation of the synlophe is oblique in *Dessetostrongylus* but frontal in *Sutarostrongylus*. Humphery-Smith (1983) by contrast, placed greater emphasis on the frontal orientation of the synlophe of *Austrostrongylus* and derived it directly from an ancestral state resembling that found in *Woolleya sprenti*.

Cassone *et al.* (1986), studying new species of *Woolleya* and *Patricialina* from dasyurid marsupials confirmed the direct *Woolleya* - *Austrostrongylus* relationship identified by Humphery-Smith (1983), but considered that *Dessetostrongylus* was a sister group to *Austrostrongylus*.

All studies on the evolution of the Herpetostrongylinae to date have relied on the comparative morphology of the adult nematodes and particularly on differences in the anatomy and orientation of the synlophe. The ontogenesis of larval stages is an important source of phylogenetic information in the Trichostrongyloidea (Durette-Desset 1985), but has not been exploited in the case of the Herpetostrongylinae other than in the case of *Beveridgiella pearsoni* (see Humphrey-Smith 1980), because no life cycles are known.

We decided to investigate the morphology of the various life-cycle stages of *Paraustrostrongylus ratti*, the only member of the genus to occur in a eutherian mammal (Obendorf 1979) to attempt to obtain additional information on the evolution of the synlophe of the genus. Due to its abundance in *Rattus fuscipes* and the ease with which infected rats could be obtained and kept in the laboratory, *P. ratti* was considered to

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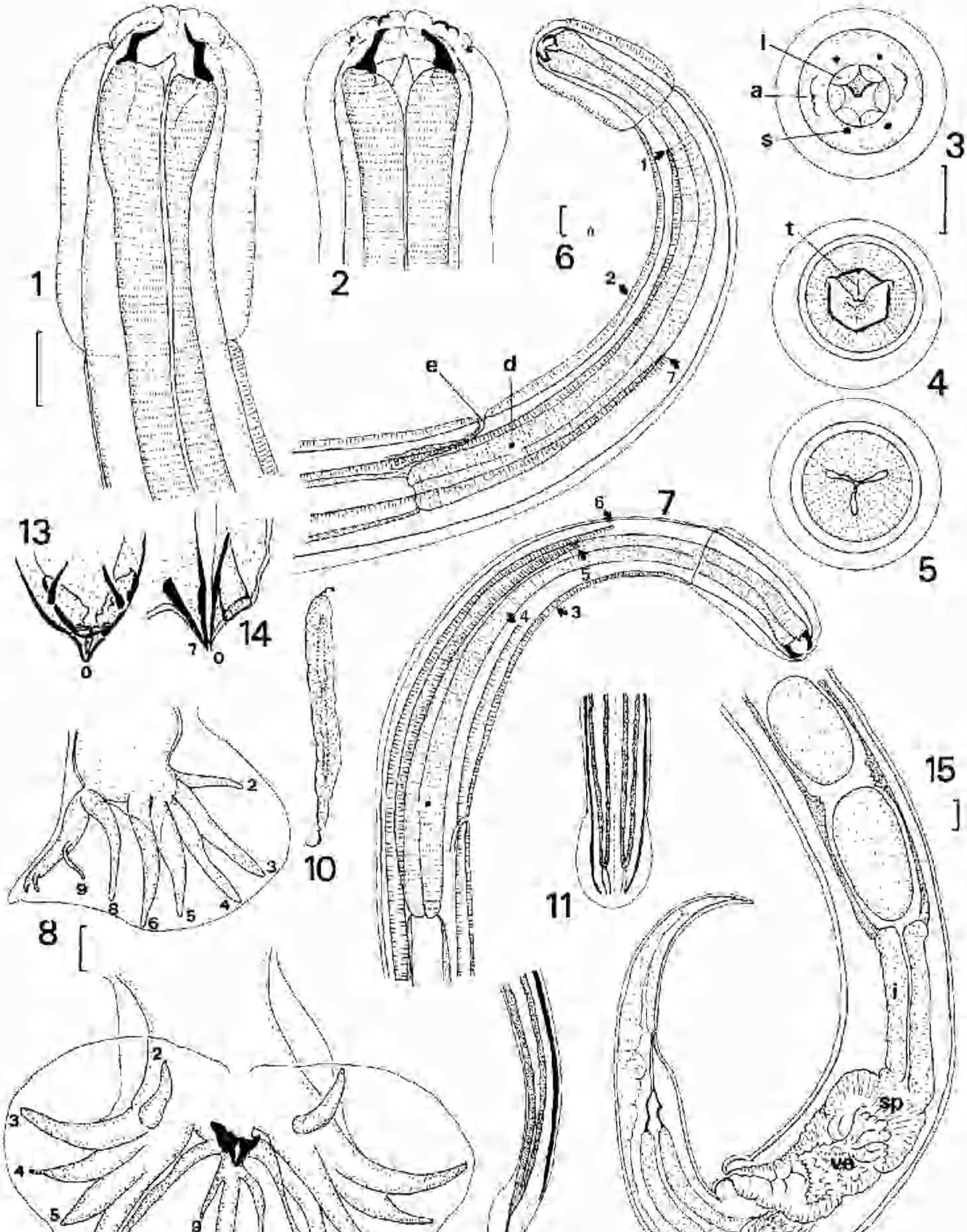


Figs 1-15. *Paraustrostrongylus ratti* Obendorf: Adult. 1, anterior end, lateral view, dorsal aspect on left hand side; 2, anterior end, dorsal view; 3, apical view of mouth opening and lips; 4 optical transverse section through hexagonal buccal capsule, with dorsal tooth; 5, transverse optical section through anterior end of oesophagus; 6, anterior region, left lateral view; arrows indicate origins of ridges; 7, anterior region, right lateral view; arrows indicate origins of ridges; 8, bursa, lateral view; numerals indicate ray numbers according to Durette-Desset (1983); 9, bursa, ventral view; 10, gubernaculum, lateral view; 11, spicule tips, ventral view; 12, spicule tip, lateral view; 13, genital cone, ventral view of papilla 0; 14, genital cone, lateral view showing papillae 0 and 7; 15, female tail, lateral view. Scale lines 0.01 mm: figs 1,2, 10, 11-14 to same scale; figs 3-5 to same scale; figs 6,7 to same scale; figs 8,9 to same scale. Legend: a, amphid; d, deirid; e, excretory pore; l, lip; p, posterior atrophic uterus; s, submedian papilla; sp, sphincter; t, dorsal tooth; ve, vestibule.

be more suitable for investigation than species occurring in marsupials. The morphological data presented here also provide the basis for subsequent ultrastructral studies.

Methods

Naturally infected rats, *Rattus fuscipes* (Waterhouse), were trapped at Blackwood, Victoria (37°29′S, 144°19′E), killed in the laboratory and the small intestine was divided into segments and opened in warm 0.09% saline. The intestinal segments were placed in an incubator for two hours to allow nematodes to migrate into the saline. Nematodes were then washed in saline and fixed in hot 70% ethanol. Small numbers of nematodes were fixed in 2.5% glutaraldehyde in phosphate buffer at 4°C.

Adult, fourth and parasitic third-stage nematodes were cleared in lactophenol and examined, using Nomarski interference contrast microscopy. Transverse sections of the body of male and female nematodes were cut using a cataract scalpel, mounted in lactophenol for examination and oriented using the methods of Durette-Desset (1971). Apical views of the anterior extremity were made by similar means. Specimens fixed in glutaraldehyde were embedded in resin. Sections cut at a thickness of 1 μm were stained with toluidine blue and were used to confirm morphological features seen in hand-cut section. Additional specimens were dehydrated in a graded ethanol series, dried in a critical point drier, coated with gold and examined with a Siemens Autoscan scanning electron microscope.

Ridges of the synlophe were numbered in an anticlockwise fashion beginning with the left-ventral ridge, in order to demonstrate homologies between stages. The numbering system for the bursal rays and papillae follows that of Durette-Desset (1985).

Faeces from naturally infected rats were collected, mixed with an equal quantity of charcoal and cultured on moist filter paper in Petri dishes at laboratory temperature. Five and eight days later, larvae emerging from the faecal-charcoal mixture were collected in distilled water and concentrated by sedimentation.

Third-stage larvae were examined live in water as well as after having been immobilised by heating. Some larvae were killed in hot 70% ethanol and cleared in glycerol by transferring to a mixture of 70% ethanol and glycerol and allowing the ethanol to evaporate.

Measurements were made either with an ocular micrometer or from drawings made using a drawing tube and are presented in the text in millimetres as the range followed by the mean in parentheses.

Morphological terminology for the synlophe follows that of Durette-Desset (1985). All drawings are oriented with the dorsal aspect uppermost and the left hand side of the nematode body towards the left margin of the page.

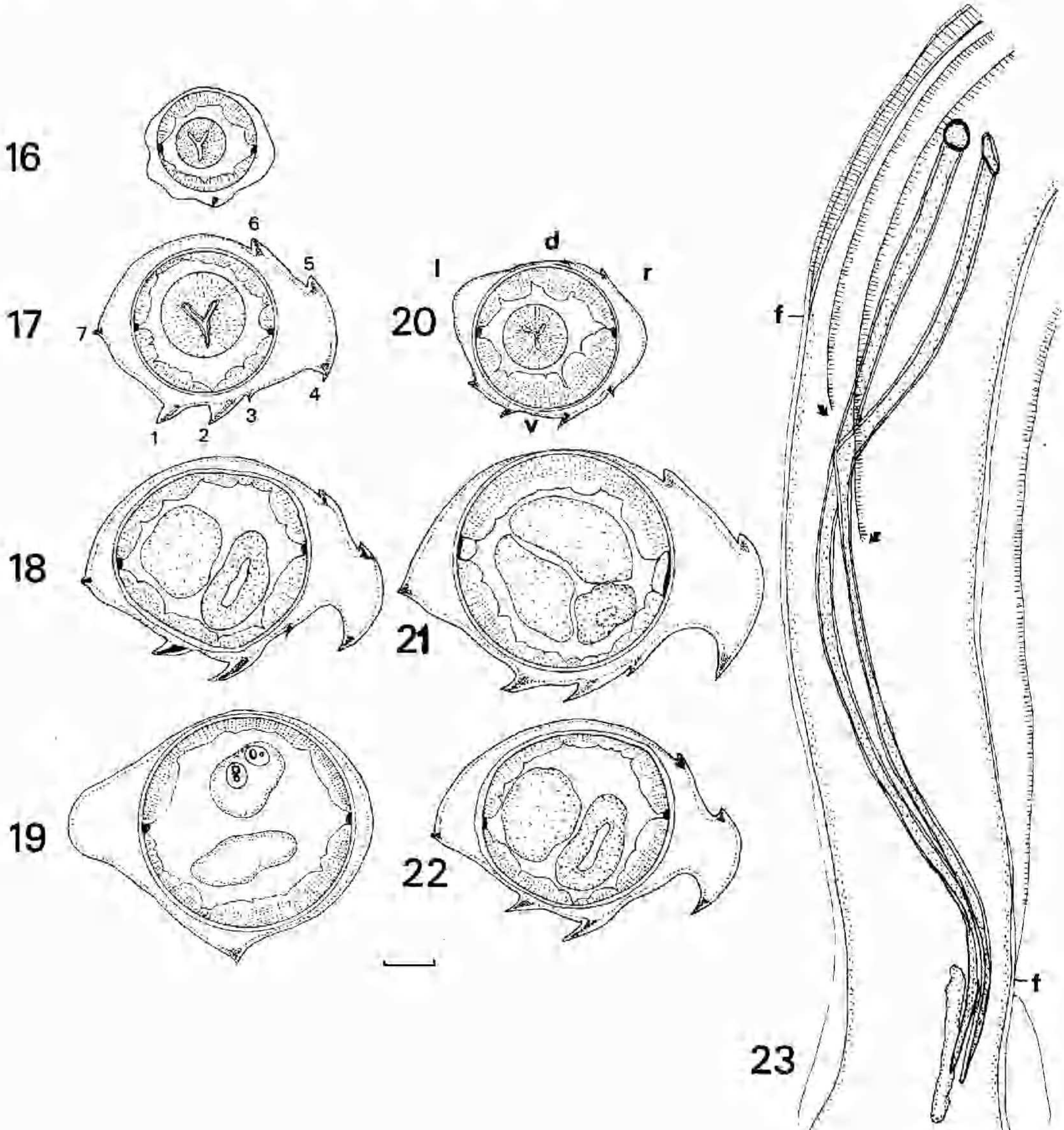
Parasitic third-stage larvae of *P. ratti* were distinguished from the synhospitalic nematodes *Nippostrongylus magnus* and *Odilia bainae* by features of the tail and cephalic extremity, based on material obtained from monospecific experimental infections with each of these two species. To obtain a monospecific infection of adult *P. ratti*, one naturally infected *R. fuscipes* was killed, all *P. ratti* in the small intestine were sorted while being maintained in warm saline, and were transferred by enterotomy to a laboratory-reared *R. fuscipes* under general anaesthesia. Four weeks later, the recipient rat was killed, the intestine removed and 10% buffered formal saline injected rapidly into it to fix nematodes *in situ*. Fragments of intestine with worms attached subsequently were dissected and prepared for scanning electron microscopy as described above. Additional segments were dehydrated; embedded in paraffin, serially sectioned at a thickness of 10 μm, and the sections stained with haematoxylin and eosin for histological examination.

Results

Adult male and female nematodes, fourth-stage larvae and two parasitic third-stage larvae were examined morphologically under the light microscope, as well as third-stage larvae cultured from faeces. Light microscopic observations were compared with scanning electron micrographs of the adults.

Paraustrostrongylus ratti Obendorf, 1979
FIGS 1-38

Description: Adult: small nematodes, red when live, spirally coiled in 3-5 tight coils, ventral surface innermost, capable of uncoiling and becoming straight when maintained in warm isotonic solutions. Cephalic extremity with prominent cephalic vesicle, 20-30 fine transverse ridges on vesicle (Fig. 1). Mouth opening, surrounded by four sub-median papillae and two amphids; no labial papillae visible; six rounded lips project into mouth opening. Buccal capsule prominently developed, heavily sclerotised, circular to sub-hexagonal in transverse section (Fig. 4), walls, arched inwards, increase in thickness towards base, lumen increases in diameter towards base; prominent tooth projects inwards from dorsal lobe of oesophagus; sub-ventral teeth absent. Oesophagus elongate, slender, clavate, widened at anterior extremity; excretory pore variable in position, usually anterior, occasionally posterior, to oesophago-intestinal junction; deirid tiny, domed, at level of excretory pore; nerve ring in mid-oesophageal region, visible in few specimens only.



Figs 16-23. *Paraustrostrongylus ratti* Obendorf: adult. 16-22, transverse sections of body. 16-19, male, 2.3 mm long; 16, at posterior end of cephalic vesicle, 0.06 from anterior extremity; 17, in oesophageal region, 0.30 mm from anterior extremity; 18, in posterior half of body, 1.60 mm from anterior extremity; 19 in cloacal region, 0.10 mm from posterior extremity; 20-22, female, 2.6 mm long; 20, oesophageal region; 21, 0.50 from anterior extremity; 22, 1.90 mm from anterior extremity; 23, posterior end of male, ventral view, showing termination of ventral ridges (arrows) and termination of floats (f). Legend: d, dorsal; l, left; r, right; v, ventral; ridges are numbered (1-7) in an anticlockwise direction from the left ventral ridge. Scale line: 0.01 mm.

Body covered with numerous fine transverse annulations (Fig. 36); two lateral, fluid-filled cavities (= floats) present on either side of body, extend from immediately posterior to vesicle to posterior region of nematode. Synlophe composed of three ventral ridges (1-3) (Fig. 36), oriented from right to left, diminishing in size from left to right; right float with two dorsal (5,6) (Fig. 35) and single ventral (4) ridge directed towards left dorsal; ridges 1-3 commence posterior to vesicle; left float, with single ridge (7) (Fig. 35) directed perpendicular to body, commences posterior to mid-oesophagus; right float with two dorsal (5,6) and one ventral (4) ridge; ridge 6 commences posterior to vesicle, followed by 5 then 4 in mid-oesophageal region.

Male (measurements of 10 specimens). Total length 1.98-2.66 (2.30); maximum width (without floats) 0.050-0.070 (0.057); cephalic vesicle 0.055-0.065 (0.064) long, oesophagus 0.27-0.30 (0.28) long, nerve ring *circa* 0.15 from anterior extremity; excretory pore 0.21-0.29 (0.25) from anterior extremity; deirid 0.22-0.30 (0.26) from anterior extremity; spicules 0.23-0.32 (0.27) long; gubernaculum 0.025-0.045 (0.037) long. Synlophe: ventral ridges and right float terminate near anterior extremity of spicules; left float continues to level of gubernaculum. Bursa symmetrical, lobes indistinct, dorsal lobe not separated from lateral lobes; rays 2 to 6 of bursa grouped in pattern of 3-2 (*sensu* Durette-Desset 1983) or 1-2-2 allowing for highly divergent ray 2; ray 2 slender, short, divergent, not reaching margin of bursa; rays 3 to 5 more robust, directed postero-laterally, of approximately equivalent size, not quite reaching margin of bursa; ray 6 shares common origin with ray 5, robust, blunt, directed posteriorly, reaches margin of bursa; dorsal trunk separate from lateral trunk; ray 8 arises from dorsal trunk, slender, does not reach margin of bursa; rays 9 slightly asymmetrical, short, slender, arise close to origin of ray 10; ray 10 stout, divides near extremity into 4 branches; outer pair of branches more robust; final branches do not reach margin of bursa. Spicules simple, elongate, alate; anterior extremities irregularly knobbed, distal tips joined; each spicule with fine, spiniform ventro-lateral branch arising in distal 1/6-1/7 of spicule; spicule tips surrounded by expanded sclerotised flange in dorso-ventral view; each main branch of spicule terminates in two fine spiniform projections within flange; gubernaculum elongate, rectangular in dorso-ventral view, composed of two layers; genital cone heavily sclerotised, complex, conical in shape, c. 0.020 long, base 0.020 wide; papilla 0 at tip of ventral lip of genital cone; paired papillae 7 on dorsal lip of cone.

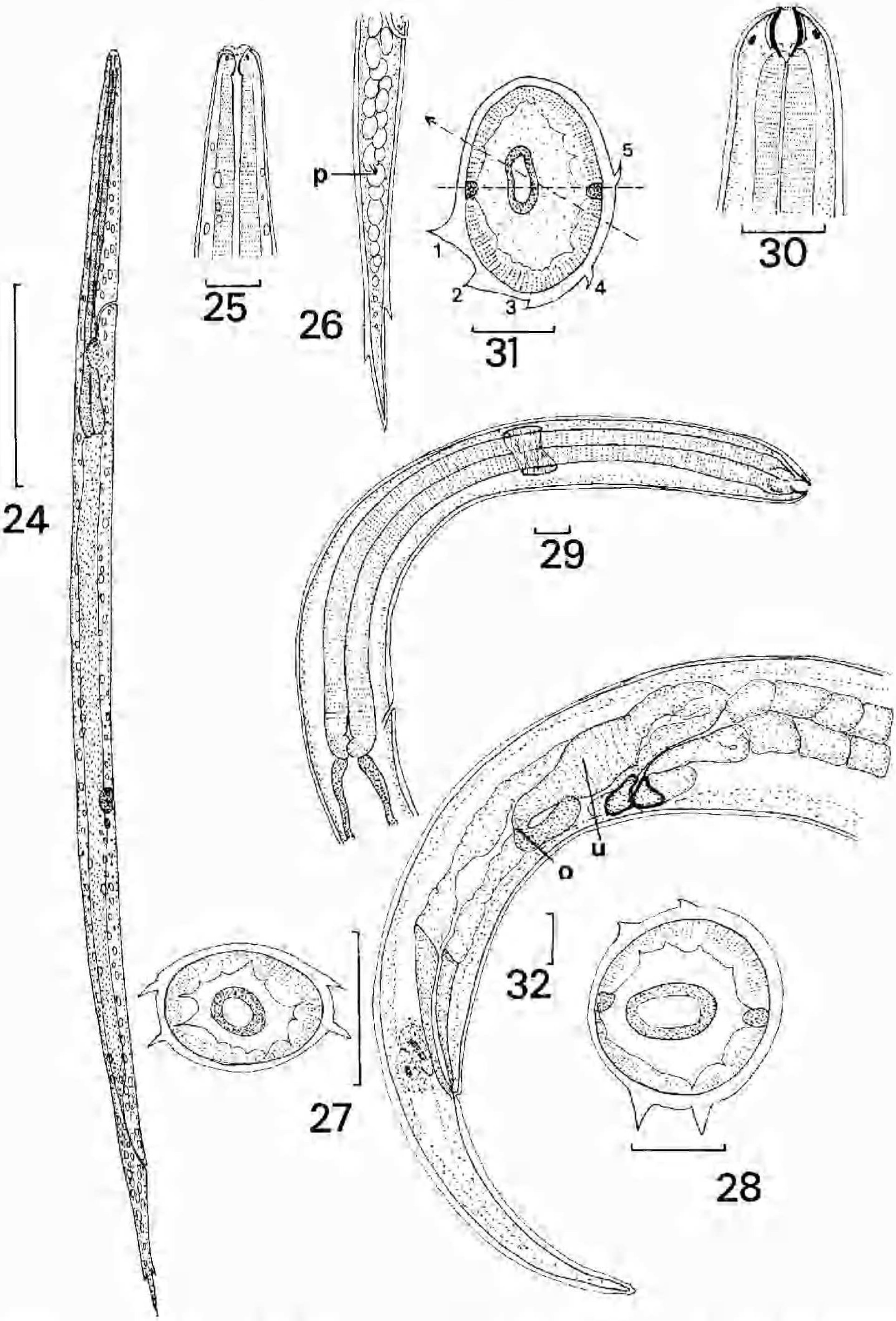
Female: (measurements of 10 specimens). Total length 2.48-2.95 (2.70); maximum width (without floats) 0.060-0.070 (0.066), with floats *circa* 0.10; cephalic vesicle 0.060-0.070 (0.063) long, oesophagus 0.26-0.32 (0.29) long; nerve ring *circa* 0.18 from anterior extremity; excretory pore 0.21-0.27 (0.23) from anterior extremity; deirid 0.22-0.26 (0.24) from anterior extremity; tail 0.08-0.11 (0.10) long; vulva to posterior extremity 0.14-0.21 (0.17); egg 0.065-0.080 (0.074) by 0.035-0.045 (0.038). Synlophe: ventral ridges extend to vulva; floats disappear in region of uterus, approx, 0.44 from tail. Tail extremely long, conical; vulva immediately anterior to uterus, opening to exterior on slight prominence; female genital system monodelphic though with posterior uterus patent and ovary persisting in vestigial form; vagina, vestibule and sphincter *circa* 0.06 long; infundibulum short, *circa* 0.04 long, prodelphic, leads to elongate uterus containing 1-4 eggs; eggs thin-shelled, ellipsoidal.

Fourth stage larva: Small nematodes, spirally coiled in 3-4 coils, ventral surface innermost; cephalic vesicle absent; mouth opening surrounded by four submedian papillae and two amphids; lips absent. Buccal capsule sub-cylindrical, heavily sclerotised, teeth absent. Oesophagus elongate, clavate; nerve ring in mid-oesophageal region; excretory pore in region of oesophago-intestinal junction. Synlophe: body floats absent; five ridges; three ventral ridges (1-3), oriented from right to left, diminishing in size from left to right; ventral ridge (4) on right hand side, oriented from right to left; single dorsal ridge (5) on right hand side, oriented dorsal from right to left; orientation of synlophe oblique from right ventral to left dorsal at about 60° to sagittal axis. Tail elongate, conical.

Male (measurements of five specimens). Total length 1.09-1.76 (1.44), maximum width 0.033-0.046 (0.040); oesophagus 0.21-0.28 (0.26), tail 0.049-0.085 (0.066).

Female (measurements of five specimens). Total length 1.47-2.00 (1.80), maximum width 0.030-0.052 (0.044), oesophagus 0.25-0.29 (0.27), tail 0.052-0.143 (0.082). Specimens with developed genitalia show distinct posterior uterus, recurving into short ovary (Fig. 32).

Third stage larva: *Parasitic:* Two parasitic third stage larvae were recorded, but detailed morphological comparisons were possible from one only. Small nematode, 0.76 long, spirally coiled in three coils; buccal capsule cylindrical, very lightly sclerotised; oesophagus slender, clavate, 0.18 long; excretory pore in posterior oesophageal region, 0.12 from anterior extremity; tail elongate, conical, with dorsal and ventral projection. Synlophe composed of two pairs of alae beginning on lateral aspects of body, perpendicular to body surface; towards mid-region of body, alae gradually shift in position to dorsal and ventral; in mid body region, the left ventral pair are larger with one in almost a mid-ventral position, and second ridge to



Figs 24-32. *Paraustrostrongylus ratti* Obendorf: larval stages. 24-27 third larval stage cultured *in vitro;* 24, entire larva, lateral view; 25, cephalic extremity, lateral view; 26, tail, lateral view; 27, transverse section in mid body region; 28, transverse section of parasitic third-stage larva from small intestine; 29-32, fourth-stage larva; 29, anterior end, lateral view; 30, buccal capsule, lateral view; 31, transverse section of larva in mid-body region, arrow indicates orientation of synlophe; 32, tail of female fourth stage larva with posterior branch of genital system. Scale lines: fig. 24, 0.1 mm; figs 25-32, 0.01 mm; figs 25-26 to same scale. Legend: p, phasmid; u, posterior uterus and o, ovary; ridges are numbered in an anticlockwise direction from the left-ventral ridge.

one side of it; two dorsal alae smaller, one almost dorsal in position, other to one side of it.

Free-living: (measurements of five specimens). Slender, elongate larvae, 0.50-0.55 (0.53) long, maximum width 0.016-0.021 (0.018); buccal capsule cylindrical, c. 0.005 long, 0.002 wide, continuous with sclerotised internal lining of anterior oesophagus; oesophagus slender, 0.14-0.16 (0.15) long; nerve ring 0.078-0.083 (0.082) from anterior end; excretory pore 0.086-0.099 (0.096) from anterior end; intestinal cells filled with granules, number of cells not ascertained; genital primordium ovoid, 0.008-0.013 (0.010) by 0.006-0.008 (0.007), 0.28-0.33 (0.31) from anterior extremity; tail elongate, conical, 0.065-0.089 (0.075) long, with dorsal and ventral spike close to tip; larva with four longitudinal alae, two on each side of body, in lateral position, almost perpendicular to body wall.

Attachment to the intestinal mucosa

Adult nematodes are coiled ventrally around intestinal villi (Figs 33, 37) usually with the tail near the distal tip of the villus; when fixed, the coils of the body are maintained (Fig. 34). At the site of attachment, nematodes compress the villi (Figs 37, 38) and, although they generally retract from the site of attachment when fixed, the impressions of the ventral ridges remain in the intestinal epithelium (Fig. 38). Changes in the epithelium at the site of attachment include cuboidal to squamous epithelial cells, elongation (= flattening) of nuclei associated with the change to a squamous cell type, loss of cytoplasmic differentiation and the loss of the brush border of microvilli. Although sometimely squamous, no defects were detected in the epithelium. No marked inflammatory changes were detected but there were significant numbers of mononuclear cells, macrophages and lymphocytes, present in the lamina propria together with a small number of eosinophils.

Discussion

Morphology of the adult

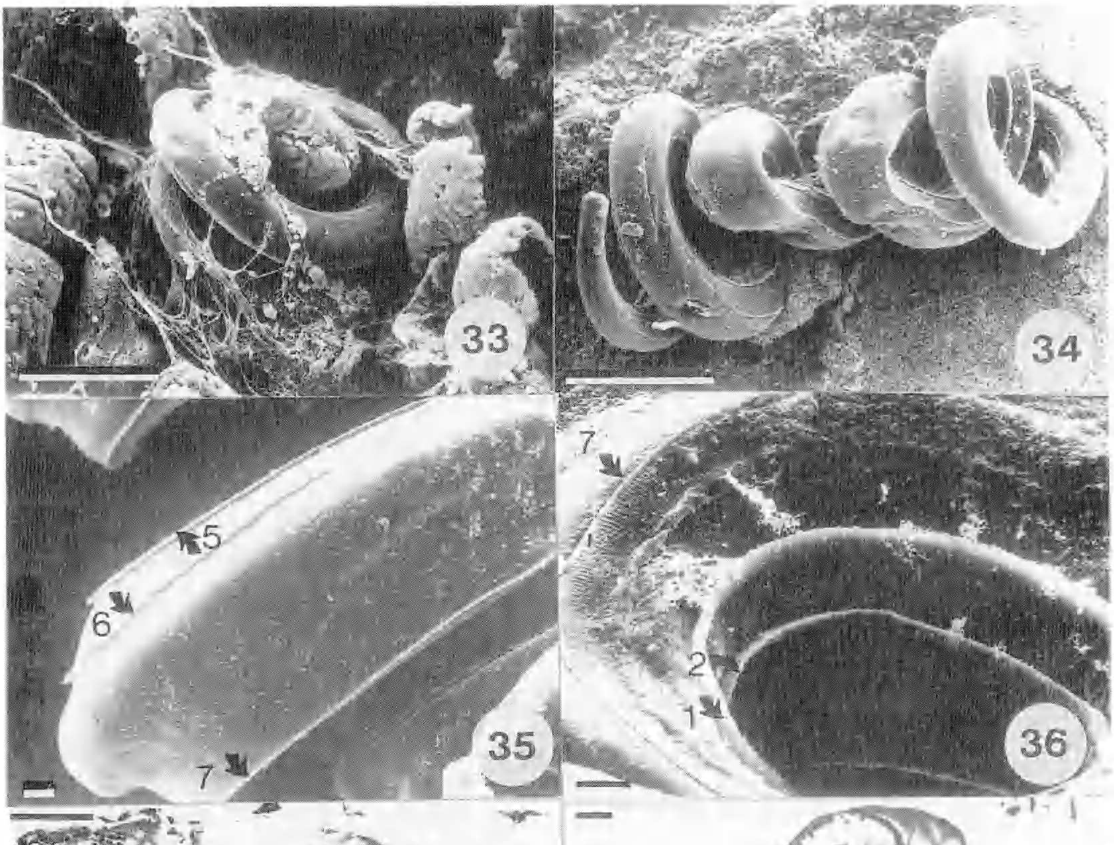
The description of the adult provided here supplements the original description by Obendorf (1979), which was found to be accurate in all essentials. Obendorf (1979) however, did not provide an apical view of the anterior extremity and provided only a single, unoriented drawing of the synlophe of the adult. In the present examination, the synlophe is described in detail, including the origins and terminations of the body ridges. Durette-Desset (1979) and Beveridge & Durette-Desset (1986) have shown that the number of body ridges changes in the posterior region of the body in several species of *Austrostrongylus* and *Paraustrostrongylus*, but comparable data were lacking for *P. ratti*. This study demonstrated that in *P. ratti*, the principal ridges arise in the oesophageal region and persist to the level of the spicules in the male and to the level of the vulva in the female. In the male, the left, but not the right float extends almost to the level of the bursa, as it does also in *P. trichosuri* and in *A. sufestatus*, the latter being a species which possesses only one float. *P. ratti* also resembles *A. sufestatus* in having three rather than the four ventral longitudinal ridges present in most other members of these genera.

The three principal ventral ridges arise close to the cephalic vesicle, while the ridges on the floats arise somewhat more posteriorly, with the ridge on the left float arising mid-way between the vesicle and the excretory pore and the two dorsal ridges on the right float arise in the anterior oesophageal region. The ventral ridge arises midway between the cephalic vesicle and the excretory pore. The origins and terminations of ridges have been reported in few confamilial species, but have been shown to be of considerable taxonomic use at the species level in genera such as *Nematodirus* (see Lichtenfels & Pilitt 1983). Preliminary observations by Beveridge & Durette-Desset (1986) on species of *Austrostrongylus* suggest that this may be the case in the Herpetostrongylinae, but features have been described in too few species to allow any firm conclusions to be drawn.

An interesting feature of the morphology of *P. ratti*, noted by Obendorf (1979) was the presence of a vestigial posterior branch to the female genital system. His observations were confirmed in this study, and the same structure was also seen in the fourth larval stage. The genera *Austrostrongylus* and *Paraustrostrongylus* are considered to be very closely related but can be separated on the basis of the presence of a sclerotised genital cone in *Paraustrostrongylus*, the absence of ventral teeth in *Paraustrostrongylus* and the position of ray 2 (Beveridge & Durette-Desset 1986). Species of *Paraustrostrongylus* are invariably monodelphic while most species of *Austrostrongylus* are didelphic. The evolution of monodelphy has occurred repeatedly in the Trichostrongyloidea (see Durette-Desset 1985) and the vestigial posterior uterus in *P. ratti* provides an obvious connection between the monodelphic and didelphic forms seen in these two closely related genera. In other trichostrongyloid genera such as *Neoheligmonella*, the posterior uterine branch may persist in the adult nematode, but does so only as a small collection of cells, posterior to the vulva (Durette-Desset & Cassone 1987), rather than the almost fully formed but diminutive posterior branch seen in *P. ratti*.

Attachment to villi

Results presented here indicate that *P. ratti* attaches to intestinal villi by coiling spirally around them, as in certain other trichostrongyloid nematodes (Durette-Desset 1985). The ventral body ridges clearly press into the intestinal epithelium, and may therefore assist the nematode in maintaining its attachment to the villus. The ventral surfaces of both body floats are also in close apposition to the epithelium and effectively increase the surface area of the nematode in contact with the intestinal epithelium. The ventral ridge of the right float (4) and to some extent the latero-dorsal ridge (4) of the right float (5) also cause indentation of the epithelium, and may therefore also assist in attachment. Once in place on a villus, dorsal ridges would seem to have little function in attachment, and one of the features of *P. ratti* is that it has few dorsal ridges. However, when observed in warm isotonic solutions, the nematode is capable of uncoiling completely, and evidence from the localisation of experimentally transplanted nematodes indicates that they are capable of migration within the intestine as is the case with



Figs 33-38. *Paraustrostrongylus ratti* Obendorf. 33-36, scanning electron micrographs. 33, entire nematode coiled spirally around villus in small intestine; 34, entire nematode, ♀, showing body coils (4); anterior end to left; 35, dorsal surface of nematode showing ridges 5 and 6 of dorsal aspect of right body float and ridge 7 on lateral aspect of left float; 36, ventral surface of posterior region of body showing ridge 7 on left body float and ventral ridges 1 and 2; 37, longitudinal section through villus showing posterior part of nematode coiled around villus, with anterior end extending to left of field; 38, histological section through villus at point of attachment of *P. ratti*, with nematode retracted, leaving sites of indentation (arrows) of ridges in epithelium. Scale bars: figs 33, 34, 37, 0.1 mm; figs 35, 36, 38 0.01 mm.

other trichostrongyloids (see Croll & Ma 1977). The dorsal ridges may therefore be of use during nematode migrations within the small intestine, when they are uncoiled and are moving between villi.

Morphology of Larval stages

The morphology of the buccal capsule of the fourth stage of *P. ratti* resembles very closely that of the fourth stage of *B. pearsoni* (see Humphery-Smith 1980) in peramelid marsupials and *Globocephaloides trifidospicularis* (see Beveridge 1979), a species parasitic in macropodid marsupials. The Globocephaloidinae, to which the latter genus belongs, was placed within the Herpetostrongylidae by Durette-Desset (1983) based on features of the bursa and buccal capsule. Since members of the sub-family lack a synlophe, their precise affinities have not been established. However, the similarities between the fourth stage larvae of *G. trifidospicularis*, *B. pearsoni* and *P. ratti* provide additional evidence that the two subfamilies are related.

Only two parasitic third larval stages of *P. ratti* were found. However, they were identified by the characteristics of the tail, which was identical to that of larvae cultured from faeces. Both parasitic larvae were spirally coiled, as are the fourth stage and adult. The pairs of alae which were clearly lateral in position at the anterior and posterior extremities of the body were slightly shifted in position in the mid-body region so that the larger, left pair were almost ventral in position, while the smaller, right pair became almost dorsal in position. This gradual shift in ridge position and the hypertrophy and ventral shift of one pair of alae presumably aids in attachment, as is the case in the adult. Two pairs of lateral alae were evident in the free-living third larval stage but they remained in the lateral position throughout the length of the nematode body. Several other trichostrongyloids have paired lateral alae in the third stage larva (Eckert & Schwarz 1965; Durette-Desset & Cassone 1987). The apparent change in orientation of the ridges in the mid-body region during the initial parasitic phase of the life cycle appears to assist the nematode in attaching to villi, but the mechanism by which this might occur is unclear. Usually there is no change in the synlophe unless a moult occurs, but in this instance, the change in position of the alae is visible in the entire nematode as well as in sections. Additional observations are clearly needed to confirm the results reported here.

Evolutionary relationships

The description of the fourth larval stage of *P. ratti* provides additional insight into the evolution of the Herpetostrongylidae. The type of information which can be provided by the larval synlophe has been discussed by Durette-Desset (1985). Generally, the larval synlophe demonstrates primitive features compared with that of the corresponding adult and frequently resembles the adults of other, related genera thereby allowing phylogenetic reconstructions. In the case of *P. ratti*, as in other trichostrongyloids, the larval synlophe differs markedly from that of the adult. The larva lacks floats; in the larva, the axis of orientation is oblique rather than being frontal, as occurs in the adult and the synlophe has two fewer ridges, lacking those found on the left float and on the dorsal right float of the adult. The larval synlophe of *P. ratti* most closely resembles that found in the adults of *Sutarostrongylus* and *Dessetostrongylus* but differs from that of *Sutarostrongylus* in having one rather than two dorsal ridges, and in orientation since the synlophe of *Sutarostrongylus* has a frontal orientation, similar to that of the adult *P. ratti*. The oblique orientation of the synlophe of the larval stage of *P. ratti* resembles that of *Dessetostrongylus*, from which it differs only in having fewer dorsal ridges. The homology of ridges between adult and larva here considered to be most likely is that ridges 1 to 3 of the larva correspond with ridges 1 to 3 of the adult, while the two additional ridges of the larva (4 and 5) correspond to two of the ridges on the right float of the adult (4 and 5). An alternative possibility would be that the first four ridges from left to right correspond to the four ventral ridges present in most species of *Austrostrongylus* and some species of *Paraustrostrongylus*, with the fifth ridge corresponding to one of the ridges on the right float. This interpretation involves postulating the loss of a ventral ridge in adult *P. ratti* and the appearance of two rather than one new ridge for the right float. The more parsimonious of the two hypotheses has been chosen here. It is also consistent with the hypothesis of Humphery-Smith (1983) that species of *Woolleya* with three left-ventral, obliquely oriented ridges, were the likely ancestors of *Dessetostrongylus*.

The suggested intermediate forms in the proposed transition series for the evolutionary lineage between *Woolleya* and *Austrostrongylus/Paraustrostrongylus* have been *Beveridgiella* (see Durette-Desset 1982; Humphery-Smith 1983) and *Dessetostrongylus* (see Beveridge & Durette-Desset 1986). *Patricialina*, an additional possibility, has a frontally symmetrical synlophe, while both *Beveridgiella* and *Dessetostrongylus* have an oblique orientation to the synlophe. *Patricialina* was considered to have been derived from *Beveridgiella* by Humphery-Smith (1983) and Cassone *et al* (1986). *Beveridgiella* has a greater number of dorsal ridges than *Dessetostrongylus*, and increases in the number of ridges occur in a number of evolutionary lineages within the Trichostrongyloidea (Durette-Desset 1985), suggesting that the synlophe in species of *Beveridgiella* is probably derived from a *Dessetostrongylus*-like ancestor. This in fact is shown in the larval stage of *B. pearsoni* (see Humphery-Smith

1980), which has a synlophe close to that of *Dessetostrongylus*. The synlophe of the fourth-stage larva of *P. ratti* differs from that of adult *Dessetostrongylus moorhousei* only in lacking an extra dorsal ridge. Because of the close correspondence between their synlophes, it appears likely that *Paraustrostrongylus* evolved from an ancestor resembling contemporary species of *Dessetostrongylus*, thus supporting the hypothesis proposed by Beveridge & Durette-Desset (1986) and Cassone *et al.* (1986).

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